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BY

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Reprinted from JOURNAL OF AGRICULTURAL RESEARCH
Vol. XV, No. 3 : : : : Washington, D. C., October 21, 1918

21 JUL 1921

PUBLISHED BY AUTHORITY OF THE SECRETARY OF AGRICULTURE, WITH THE COOPERATION OF THE ASSOCIATION OF AMERICAN AGRICULTURAL COLLEGES AND EXPERIMENT STATIONS

WASHINGTON: GOVERNMENT PRINTING OFFICE: 1918

ANGULAR-LEAFSPOT OF CUCUMBER: DISSEMINATION, OVERWINTERING, AND CONTROL

COOPERATIVE INVESTIGATIONS BETWEEN THE UNIVERSITY OF WISCONSIN AND THE BUREAU OF PLANT INDUSTRY, UNITED STATES DEPARTMENT OF AGRICULTURE

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INTRODUCTION

The bacterial nature and the symptoms of the angular-leafspot of cucumber (*Cucumis sativus*) have been clearly described by Smith and Bryan (*15*).[2] Prior to this paper the disease had been reported in this country by Burger (*1–4*) and from Europe by Traverso (*16*) and Potebnia (*12*). The two latter writers accepted Burger's statement that the organism which caused the spots on the leaves was also responsible for a serious rotting of the fruit. The inoculation studies made by Smith and Bryan (*15*) showed that the bacterium which caused the leaf-spotting was unable to produce a soft-rotting of the fruit. Extensive tests by the writer have confirmed their finding in this regard.

The bacterial cause of the disease was determined independently by the writer in the summer of 1915, as set forth in a preliminary note (*5*). The morphological and physiological studies which were subsequently made of a strain of the causal organism isolated from a Wisconsin specimen gave results essentially in agreement with those reported by Smith and Bryan (*15*). The name given to the organism by these writers is "*Bacterium lachrymans*." According to Migula's system of classification it would be called "*Pseudomonas lachrymans*."

The damage caused by the angular-leafspot can not be accurately estimated. It varies greatly with differing weather conditions, but enough weather favorable for the disease prevails each year to make the injury of considerable importance (Pl. 13, A). The writer's ideas as to the destructiveness are based mainly on his field experience in Wisconsin and adjoining States during three summers, together with more limited observations in Virginia and southern California.

It is when the disease appears in a field early in the summer that the greatest damage results, as would naturally be expected. Young plants

[1] The writer wishes to express his appreciation to Dr. L. R. Jones, of the Wisconsin Experiment Station, for helpful interest and advice in the prosecution of the work and to thank Mr. W. W. Gilbert and Dr. M. W. Gardner, of the Bureau of Plant Industry, for helpful suggestions and cooperation.

[2] Reference is made by number (italic) to "Literature cited," p. 220.

are often so severely attacked that stunting results (Pl. 13, B). A few scattered plants in a field or nearly all may be so affected, depending chiefly on the meteorological conditions. Some observations on the extent of the injury by angular-leafspot in a representative locality may here be noted to give a more definite idea of the damage which it causes. At Ripon, Wis., in the summer of 1914, sixteen cucumber fields were under observation. The disease appeared in seven of these while the plants were in the seedling stage, and by the middle of the season, August 11, it had resulted in the severe spotting of approximately 25 per cent of the leaves. On August 11 the disease was also present in three of the nine other fields, but in these it had been introduced only a short time and had not yet become generally distributed. A survey of the same locality on August 15, 1915, indicates how widespread the disease may become, especially when the fields are close together. On that date 28 out of 35 fields visited were found to be infested. A later visit revealed even further distribution.

The losses in the regions where cucumbers are grown for pickling purposes result mainly from the decrease in yields due to the destruction of leaf surface, but it seems quite probable that in other sections, as has been pointed out by Burger (*1*, *2*) for Florida, where cucumbers are grown for "slicing" purposes, and so must be shipped to distant markets or kept in storage for considerable lengths of time, an additional loss may come from the secondary soft-rotting of the fruit. Limited observations by the writer indicate that in California the soft-rotting of the fruit as an indirect result of the angular-leafspot may cause some loss. The bacterium causing the leafspot does not directly cause the fruitrot, but through the wounds which it makes on the fruit softrot organisms are frequently able to gain entrance.

The widespread distribution of angular-leafspot and its frequent occurrence give it a place among the major diseases of the cucumber. The aggregate loss which it entails probably exceeds that caused by some of the other diseases which are more destructive in limited areas. In America this disease has been reported from Florida by Burger (*1–4*), and from Connecticut, Indiana, Michigan, New York, and Wisconsin and the Canadian Provinces of Ontario and Quebec by Smith and Bryan (*15*). To this list of regions where it is known to occur may now be added California, Colorado, Illinois, Iowa, Minnesota, and Virginia. That it is probably widely distributed in Europe is indicated by the fact that Traverso (*16*) reported it from Italy, and Potebnia (*12*) recorded its occurrence in Russia. The wide distribution of the disease is a fact that should be expected in view of the evidence to be presented that the causal bacteria are seed-borne and in view of the general occurrence of the trouble in seed-growing localities.

PRELIMINARY CONSIDERATIONS

The chief purpose of this paper is to present evidence bearing on the phases of the problem which are of direct economic significance. Certain other parts of the work which has been done have yielded results worthy of record; and, since some of these results are pertinent to the questions of dissemination, overwintering, and control, they may appropriately be presented before passing to the consideration of the latter points.

DESICCATION

Many questions in regard to the dissemination and overwintering of the causal organism of angular-leafspot depend on its sensitiveness to desiccation. The organism has been shown by repeated tests to be relatively sensitive to drying on glass. With a 3-mm. platinum loop drops were transferred to carefully cleaned cover glasses from 36-hour cultures in beef bouillon and in cucumber-leaf decoction from the leaf exudate and from a suspension in distilled water of the organisms from freshly invaded tissue. None of these showed viable organisms after four days' drying at room temperature. Smith and Bryan (*15, p. 470*) found that the organisms from a young bouillon culture when dried on glass were viable after 21 days. The variance in these results may possibly be due to some slight difference in methods, which may have made a difference in the time for which the bacteria were exposed to complete desiccation.

Freshly invaded fruit and leaf tissue dried in diffuse light at room temperature showed viable organisms after 3 and 10 days, but none were alive after 32 days.

Short periods of drying, four to five days, resulted in the death of all organisms on seed which had been disinfected with mercuric chlorid, washed thoroughly, and then wet with a young bouillon culture of *Bacterium lachrymans*. The fact, however, that the bacteria do survive long periods of desiccation on or in the seed is shown by the evidence to be presented under the discussion of overwintering.

The results of one test on culture media are here pertinent because they show that there are conditions under which the organisms may withstand long periods of drying. On February 2, 1916, six tubes of potato-dextrose agar, in each of which had been suspended approximately 0.5 gm. of powdered calcium carbonate, were slanted and inoculated. The purpose was to see if the life of the cultures might be prolonged by neutralizing with the carbonate the acid resulting from the growth of the organisms. In a dextrose-containing medium the bacteria ordinarily make a rapid growth for a short time and then all die, so that the tubes become sterile, usually within 10 days. The tubes in this test were set away at room conditions and, because of the low relative humidity of the laboratory air, rapidly dried out. Before they dried com-

pletely, which required nearly two months, an abundant growth of the organisms had been made. On November 8, 1916, the dry remains of the agar, carbonate, etc., from three of the tubes were transferred to tubes of bouillon. Growth occurred in all three tubes thus inoculated, and in each case the identity of the organism was established by inoculating cucumber plants. Two of the remaining three tubes were similarly tested on February 9, 1917. Growth resulted in each case, and inoculations again proved that the clouding of the bouillon was due to the angular-leafspot organism. Since all other evidence is opposed to the possibility of the formation of spores, the writer is inclined to explain the survival of some of the bacteria in these tubes by assuming that they were protected from complete desiccation.

THERMAL RELATIONS

The thermal death point of the angular-leafspot bacterium is between 49° and 50° C. Tests were made in 10-cc. portions of beef bouillon in thin-walled test tubes at 46°, 48°, 49°, 50°, 52°, and 55°. Ten minutes' exposure at 46° must have killed a large proportion of the organisms, because growth in tubes so exposed was much slower in appearing than in the unheated controls. In each test some but not all of the tubes exposed at 49° C. showed no growth. In none of the tests did growth occur in tubes exposed at 50° or temperatures above that point.

An interesting contrast between the relation of temperature to angular-leafspot and its relation to the bacterial-wilt of cucumber was brought out at Madison, Wis., in 1916. The maximum temperature as recorded by the United States Weather Bureau there averaged 36.7° C. (98° F.) for the five days July 26 to 30, inclusive. The highest temperature at the Weather Bureau Observatory was 38.3° C. (101° F.), but in direct sunlight and near the ground undoubtedly the temperature was higher. This unusually hot weather did not appreciably check the development of angular-leafspot, which reached its maximum development within about 10 days thereafter, but it practically stamped out the bacterial-wilt. Smith (*14, p. 209*) accoun s for the bacterial-wilt having been found only in cool climates on the basis of the low thermal death point, 43° C., of the causal organism.

The relation of temperature to growth in artificial media has been found to agree with the report of Smith and Bryan (*15, p. 470*), and so need not be given in detail.

The sensitiveness of the bacteria to freezing was tested by exposing them in different media in glass test tubes outside a north window during a period of low temperatures in the winter of 1916–17. Dilute suspensions of the bacteria in distilled water, freshly-inoculated tubes of beef bouillon, beef bouillon with approximately 2 per cent of sodium chlorid, and 24-hour agar slope cultures were exposed. During the first 9 days of the

exposure the highest temperature was 0° C. (32° F.), the lowest −25.5° C. (−14° F.), and the average daily mean was −15.5° C. (4.1° F.). One tube of each medium was taken in after 24 hours and longer periods and thawed slowly in cold water. In the salt bouillon all the bacteria were dead after 24 hours. In bouillon without salt all were dead after 60 hours. No test was made of the suspension in distilled water after the 60-hour interval, but no colonies developed in plates poured from one of the other tubes melted after 4 days. On the agar some of the organisms were alive after 6 days, but after 17 days all were dead. The sensitiveness to freezing was undoubtedly increased by the sodium chlorid in the bouillon.

Smith and Bryan (*15, p. 471*) reported freezing the organisms for 15 minutes in bouillon by means of salt and pounded ice. That exposure resulted in the death of nine-tenths of the bacteria.

SENSITIVENESS TO GERMICIDES

Tests of the sensitiveness of the organisms to formaldehyde, copper sulphate, and mercuric chlorid were made. The dilutions of formaldehyde were made up by volume from the 40 per cent formaldehyde solution known commercially as formalin. The copper-sulphate and mercuric-chlorid solutions were made up 1 to 1,000 by weight and the desired dilutions made from these. Exposures were made in all cases by transferring a 3-mm. loop of a young bouillon culture to 10-cc. portions of the dilutions in vials floated on a water bath at 25° C. Tubes of melted agar were inoculated in duplicate or triplicate by a 3-mm. loop transfer from each vial after an exposure of 10 minutes.

The test with formaldehyde resulted in the death of all organisms exposed to a dilution of 1 to 10,000, of nearly all in the 1 to 100,000 dilution, and of apparently none in the 1 to 500,000 dilution. The tests with copper sulphate and mercuric chlorid were repeated twice. With the copper sulphate the results did not agree throughout, but in all cases all organisms were killed or prevented from developing by the 1 to 100,000 dilution. There were no colonies, or a strikingly smaller number than from the controls, in the plates poured from the 1 to 500,000 dilution. All organisms were killed by exposure to dilutions of mercuric chlorid of 1 to 1,000,000.

The sensitiveness of the organism to copper sulphate was tested by Smith and Bryan (*15, p. 474*). Their results show a slightly less marked sensitiveness to this chemical than was found in the tests made by the writer. The temperature at which their exposures were made was not stated.

PLANTS ATTACKED

Little attention had been previously given to the question of the host range of the disease or to the question of variations in susceptibility or resistance to the disease in the case of the different types of cucumbers.

Because of the bearing which these questions might have on distribution, overwintering, and control, 12 horticultural varieties of cucumbers and a large number of other cucurbits were tested as to susceptibility to angular-leafspot. The varieties of field cucumbers and the other species and varieties of cucurbits which are listed in the following table were grown in a cucumber field thoroughly infested with angular-leafspot, where they were under the most favorable conditions for infection. The varieties of forcing cucumbers were tested by inoculation in the greenhouse.

Plants exposed to angular-leafspot infection

PLANTS ATTACKED	PLANTS NOT ATTACKED
1. Cucumber (*Cucumis sativus*), 12 horticultural varieties:	1. Balsam-apple (*Momordica balsamina*).
Davis Perfect.	2. Balsam-pear (*Momordica charantia*).
Chicago Pickling.	3. Squirting cucumber (*Momordica elaterium*).
Boston Forcing.	4. Pomegranate melon (*Cucumis melo* var. *dudaim*).
Early Russian.	5. *Cucumis grossulariaeformis*.
Giant Pera.	6. Muskmelon (*Cucumis melo*), 11 varieties.[a]
Japanese Climbing.	7. Snake melon (*Cucumis melo* var. *flexuosus*).
Heinz Muscatine.	8. Wild cucumber (*Echinocystis lobata*).[b]
Lemon.	9. Watermelon (*Citrullus vulgaris*), 2 varieties.[a]
Thorburn's Everbearing.	10. Citron (*Citrullus vulgaris*).
Rollison's Telegraph.	11. Japanese crookneck squash (*Cucurbita moschata*).
Vaughan's Prolific Forcing.	12. Hubbard squash (*Cucurbita maxima*).[a]
White Spine Klondyke.	13. Turban squash (*Cucurbita maxima*).
2. West Indian gherkin (*Cucumis anguria*).	14. Summer squash (*Cucurbita pepo* var. *condensa*).
3. Mandera gourd (*Cucumis acutangulis*).	15. Pumpkin (*Cucurbita pepo*).[a]
4. Hedgehog gourd (*Cucumis dipsaceus*).	16. Gourd (*Cucurbita pepo* var. *ovifera*).
5. Calabash gourd (*Lagenaria vulgaris*).	17. *Trichosanthes colubrina*.
6. *Bryanopsis laciniosa*.	

In the case of the plants other than cucumber attacked the causal organism was isolated from diseased spots and identified by inoculating cucumbers. Stained sections from paraffin-embedded material showed bacteria within the tissue of the leafspots in all cases except that of the West Indian gherkin, which was unquestionably subject to the disease.

STOMATAL MOVEMENT AND INFECTION

The fact that leaf infection took place through the stomata was reported by Smith and Bryan (*15, p. 469*), but they gave no discussion of the conditions necessary for infection. Practically all of the earlier inoculations made by the writer were performed in the evening, after dark,

a Greenhouse inoculations also gave negative results.

b The wild cucumber plants were not in the experimental plots, but grew near by and were artificially inoculated.

because the sensitiveness of the organism to sunlight was known and because moisture, such as dew on the leaves, was thought to be the most important factor in infection. A few infections were nearly always obtained in this way, but the number was consistently smaller than occurred on leaves naturally infected. A suggestion that the factors limiting the number of leaf infections were in some way involved with the time of inoculation was obtained when, from a series of inoculations made in the field at intervals of 2 and 4 hours during the day and night of a 24-hour period, more abundant infections resulted from the inoculations made during the day. Evidence that infection occurs more abundantly when inoculations are made during the day was confirmed by other tests.

The idea that during the process of photosynthesis enough oxygen was given off through the stomata to exert a chemotactic action on the causal bacteria was first conceived as a possible explanation of the different results from night and day inoculations. The hypothesis was abandoned after experimental tests. Plants which were kept in darkness for 24 hours before and after inoculation became infected to about the same extent as the controls.

The idea that stomatal movement might be a factor was next hit upon. Pool and McKay (*11*) found that there was a relation between stomatal movement in sugar-beet leaves and infection by *Cercospora beticola*. This fact suggested that in the case of the disease under consideration a similar relation might hold true. To study the behavior of the stomata the method described by Lloyd (*10*) of direct visual observation of the stomata *in situ* was utilized. It was found that the stomata on the lower surfaces of the leaves were generally open during the day and closed at night. The movement of the stomata on the upper surfaces was not always the same as those on the undersides, but this fact is of no special significance here. It was then found by repeated tests that inoculations on the under surfaces made in the morning, when the stomata were observed to be open, gave much more abundant infections than did similar inoculations made at night, when the stomata were seen to be closed (Pl. 14). The following table gives a comparison of the number of infections from night and day inoculations. The two plants used were of the same age, each having seven leaves at time of inoculation. Leaf 7 is the youngest leaf of each. They were treated similarly except for time of inoculation.

Time inoculated.	Leaf 3.	Leaf 4.	Leaf 5.	Leaf 6.	Leaf 7.
7.30 p. m.	0	0	5	48	45
10.00 a. m.	16	43	49	97	50

On the plant inoculated in the evening the youngest leaves, No. 6 and 7, showed many more infections than did the older leaves, and this has been repeatedly found in other inoculations. Why this difference in in-

fection of leaves of different ages occurs is a matter of conjecture, but it is thought to be associated with the fact that younger tissues are more susceptible. Probably the relatively small number of organisms which retain their motility are able, when the stomata open, to establish themselves in the younger leaves, but are not able to gain a foothold in the older tissues.

The closure of the stomata may mechanically exclude the bacteria or may interfere with stimuli which attract them into the interior of the leaves. No attempt has been made to determine this point, but the first theory seems to the writer the more plausible.

FRUIT INFECTION

Fruit infection occurs naturally without wounds. Stomatal infection (fig. 1) has been demonstrated in fruit artificially infected without wounding. Burger's (*4*) description of the effect on the fruit is accurate in part, but the softrot which he emphasizes results from organisms other than the species causing the small, circular, localized spots on the fruit, characteristic of angular-leafspot infections. The circular spots are at first water-soaked in appearance. Later their centers become whitened, owing to a cracking and drying out of the tissues (Pl. 16, B). In fruit, as well as leaf tissue, the bacteria have been seen only in the intercellular spaces.

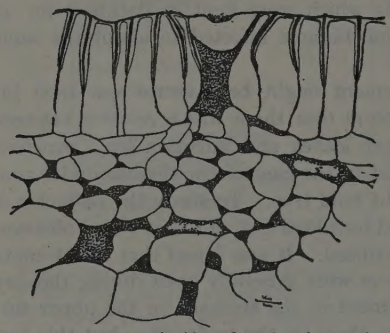

FIG. 1.—Cross section of epidermal portion of cucumber fruit fixed eight days after inoculation with *Bacterium lachrymans*, showing presence of bacteria in stoma and tissues below.

DISSEMINATION

The means by which the disease is spread have been given a good deal of attention because of the possible bearing which these might have on remedial measures. Some of the observations and experiments may throw light on other and similar bacterial diseases.

BY RAIN AND WIND

That the important relation of rainy weather to the progress of angular-leafspot, a factor previously observed, was principally in the dissemination of the causal organisms was made clear in the summer of 1916. Healthy potted plants which had been placed outside of the greenhouse and at a distance of 4 feet from infected plants became diseased after a

rainy period. Experimentally infection was secured by placing recently infected leaves on the ground beneath healthy plants on a day when there were frequent showers. In the fields at Madison newly infected spots appeared in abundance within five or six days after heavy rains, especially the rains of July 19 and August 3–5. Rain must fall at relatively frequent intervals to be effective in spreading the disease. Prolonged rainless periods check the development of the disease to a great degree, especially if accompanied by high temperatures (Pl. 15, C).

The importance of rain in the development of the disease was clearly shown at Ripon, Wis., in 1914. Owing to favorable rainy weather early in the season angular-leafspot spread throughout certain fields. Two of the infected fields at Ripon and one in a neighboring locality, which were visited on August 11 and 12, presented a striking appearance.

The vines were so grown together as to nearly cover the ground, but the centers of the rows were clearly marked by the old, angular-spotted leaves in contrast with the healthy green of the later growth which had developed after the last heavy rain.

Further evidence regarding the importance of rain in relation to the development of the disease was furnished by a comparison of conditions at Madison and Ripon, Wis., in 1916. The striking difference in the amount and distribution of rainfall for the two places during the month of July can be seen in Table I.

TABLE I.—*Dates and amounts of rainfall at Madison and Ripon, Wis., in July, 1916*

Day of month.	Precipitation (inches).	
	Madison.	Ripon.
1	0.00	0.13
12	.33	.11
16	.90	.00
19	1.21	.00
20	.19	.00
22	.03	.00
26	.00	.02
Total	2.66	.26

The time of planting and the earlier weather conditions were similar, and so it is highly probable that the disease appeared in both localities at about the same time, noted first at Madison on July 3. At the end of the month the disease was widespread and affecting leaves of all ages in the Madison fields, while at Ripon only the older leaves at the hill centers showed the angular spots.

The relation of wind to dissemination by rain spattering has not been studied experimentally, but the comparison of the way the disease spread in differently situated fields throws some light on the question.

One field, which we may call field A (fig. 2), was on the southeast slope of a hill and surrounded by trees so that it was well protected from wind, especially northwest wind. Field B was on the west slope of another hill and freely exposed to wind. The original centers of angular-leafspot in B were on the north side near the top. Thunder showers on July 12, 16, and 19 were accompanied by high winds from the north and west. On July 29 it was noted that in field A the infested areas were strikingly more delimited than those in field B. The difference could be explained only as a result of the difference in exposure to winds.

Another field was situated on a freely exposed west slope, and its rows ran with the hill, east to west. No notes on disease distribution there were taken until August 4. On that date there was a center of abundant

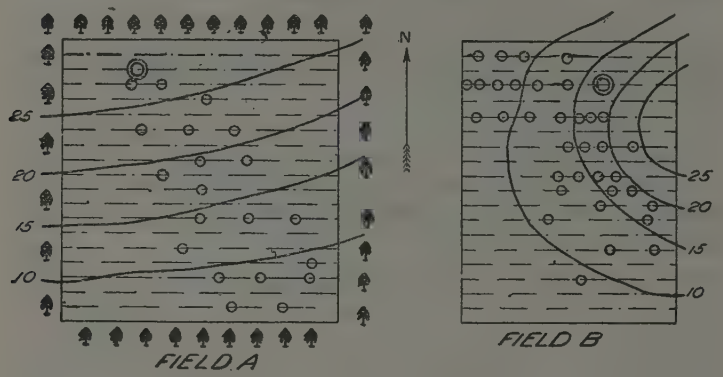

FIG. 2.—Diagrams of cucumber fields to show relation of wind and drainage water to angular-leafspot dissemination. Double circles indicate the location of original centers of infection prior to storms. Small circles represent secondary infections due to storms. In field A drainage water only was an important factor because of protection from wind while in the freely exposed field B wind also played a part. Contour lines indicate elevation above level of Lake Mendota, Madison, Wis. Broken lines show the direction of the rows. For full explanation see text.

infection in the seventh row from the north side and scattered infections in all of the 16 rows south of it. North of this badly infested area the adjacent row, the sixth, showed a very small number of infections, and the 5 others were entirely free from the disease. Obviously the northwest winds had played an important part here also in spreading the disease. Faulwetter (7) has shown that wind in connection with rain is an important factor in the spread of a similar bacterial disease, the angular-leafspot of cotton.

The fact that the thundershowers mentioned as resulting in a marked spread of angular-leafspot occurred during the daytime supports the inference which may be drawn from the facts regarding the relation of stomatal movement to infection, that rains which occur in the daytime are more effective in the spread of the disease than are those occurring at night.

BY DRAINAGE WATER

Evidence concerning the distribution of the causal organism by drainage water during rains was afforded by comparing developments in fields A and B which were mentioned in the preceding section. The rows in field A ran across the hillside, while in B they followed the direction of the slope (fig. 2). After the rains of July 12, 16, and 19 the disease appeared in field B throughout the length of the rows in which it had been noted earlier and in plots below them where disinfected seed had been planted. In field A, however, the spread of the disease was not mainly along the rows but rather crossed the rows, following the path of the drainage water. The supposition is that the organisms were carried by the drainage water and from it were spattered by the rain to the healthy plants. Dissemination by drainage water has been noted before with fungus diseases—for example, cabbage-yellows by Jones and Gilman (*9*)—but, so far as is known to the writer, no evidence has before been published in regard to its significance in the case of a bacterial disease.

Attempts to prove that drainage water carried the causal organisms were made on two occasions late in the summer. Samples of drainage water caught during rains at the lower edges of infested fields were taken to the greenhouse and sprayed on healthy plants. The negative results are not surprising in view of the fact that few new infections developed in the fields where the water was caught, and that negative results from attempts to isolate the bacterium from beetles from these same fields also indicated that a large proportion of the bacteria had been killed as a result of the long, preceding period of dry weather.

BY PICKERS

The spread of diseases due to fungi has been attributed to pickers—for example, bean anthracnose, by Whetzel (*17*)—but, so far as the writer is aware, no such fact has been demonstrated for a bacterial disease. Experiments in the case of the cucumber angular-leafspot have shown that the disease may be spread by pickers if picking is done when the exudate is present on the infected leaves. On August 8 and 9, 1916, the matter was tested as follows: At 5.30, 7.30, and 8 o'clock on the morning of the first day and at 8 a. m. on the second day two or three leaves in each case were inoculated by rubbing with the hands (as is done by pickers) after having first rubbed them through the exudate on diseased leaves. In all four cases inoculated leaves became infected (Pl. 15, B), while the uninoculated controls remained healthy.

Picking is, of course, frequently done early in the morning and on rainy days when the leaves are wet and the bacterial exudate is abundant. Numerous observations show that the spread of the disease in the way described in the preceding paragraph often results. The most obvious

of the cases that have come under the writer's notice seems worth mentioning in detail. At Princeton, Wis., a patch of cucumbers of seven rows was visited on August 12, 1916. In the middle of the third row, counting from the north side, there was a circular area of diseased leaves, badly shattered by the rain of August 10. West of this area of shattered leaves no new infections were evident. East of this area, however, there were numerous recent infections, and the number of these varied nearly inversely as the distance from the original center. The location of diseased leaves and the position of the spots on them corresponded to observations on dissemination by pickers made in other places. When passing the patch later in the day the owner was seen starting to pick on the west end of the first row, so that when he would come to pick the third row he would be working eastward (fig. 3). Evidently he must have followed the same course when the bacterial exudate was abundant enough to thoroughly contaminate his hands.

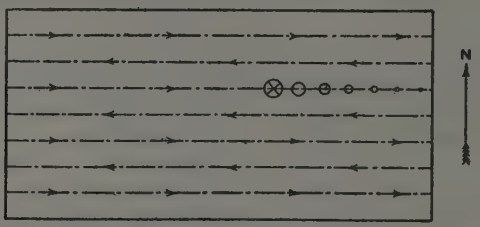

FIG. 3.—Diagram of cucumber field to illustrate picker dissemination of angular-leafspot. ⊗=original center, ○=new infections, arrows indicate direction the picker worked.

BY INSECTS

Cucumber beetles (*Diabrotica vittata* Fab. and *D. duodecimpunctata* L.) have repeatedly been seen crawling over infected leaves and flying about the fields when the bacterial exudate was plentiful, as early as 5.30 a. m. Platings from these insects were made in only a few cases. That some of them would crawl through exudate and become contaminated seemed unquestionable. In one instance platings from a water blank, in which had been dropped three 12-spotted beetles, yielded the causal organism.[1] Bees have been observed visiting the plants as early as 7.30 a. m. and have been seen to occasionally brush against exudate-bearing leaves.

The carrying of the causal bacteria from one part of a field to another by insects is no doubt significant, but in view of the other ways in which the local spread is accomplished it is far less important than is the dissemination of the organism by the same agency from diseased to healthy

[1] The organism in this case was not tested as to pathogenesis, but was identified by colony characters on potato-dextrose agar. These typical colonies were more numerous than any other kind, and by transfers (unintentionally delayed until 10 days had passed) it was shown that the organisms were dead, as is true for the angular-leafspot organism with media containing dextrose.

fields. The evidence supporting the latter idea is observational. During 1916 six experimental fields were grown near Madison in the same vicinity with four privately owned commercial fields. The distances between the commercial and the experimental fields varied from about 30 rods to ½ mile. Angular-leafspot appeared in all six of the experimental plots early in July. It appeared in only three of the others and in these not until nearly the middle of August. Prior to this, in the forepart of the month, there was a period in which there was an abundance of bacterial exudate in the infested fields and when insects, especially the beetles, were very active. It is of interest here to note that in no case did the original center of infection in a private field develop at the edge, but rather in the interior of each of the three patches. It may be stated with confidence that during the time in question no one except Dr. M. W. Gardner and the writer visited both the experimental and commercial fields, and as these visits were made when the leaves were dry there seems little probability of the organisms having been transported by us. Comparable developments were observed at Ripon, Princeton, and Pittsville, Wis.

Closely correlated with picker dissemination and the probability of spread by insects is the relation of atmospheric humidity to the disease. Under conditions of high relative humidity, such as frequently prevail on summer nights, the invaded areas of the leaves take on clear-cut angular shapes and the bacterial exudate becomes abundant. (See Pl. 13, A). Such nights were those of August 8 and 9, 1916. The relative humidity, as recorded by a Friez hygrograph, varied on those nights from 74 and 80 per cent, respectively, at 7 p. m. to 90 per cent, where it continued until 6 a. m. Observations in the early mornings showed abundant signs, as described, that conditions for the progress of the disease had been most favorable. In steam-heated greenhouses, with the relative humidity varying from 45 to 60 per cent, the disease develops poorly or not at all.

OVERWINTERING

The several ways in which the causal organisms of other bacterial diseases have been thought to pass the winter have been kept in mind in searching for evidence as to how the angular-leafspot bacterium overwinters.

SOIL

The sensitiveness of the organisms to freezing, as elsewhere recorded, renders doubtful the possibility of their living over the winter in the soil, or in the débris of diseased vines in northern climates. A limited amount of work on this question indicates that the bacteria do not live for long periods in the soil. The question can not, however, be definitely settled until further study of it has been made.

INSECTS

The hypothesis that the bacteria may overwinter in or on the bodies of insects is here mentioned because it might seem plausible in view of the theory advanced with good evidence by Rand and Enlows (*13*) to account for the overwintering of the organism causing the wilt of cucurbits. No dependent relationship, such as has been found to exist between the wilt and cucumber beetles, has been observed in the case of angular-leafspot. Field observations in 1916 furnish some good negative evidence relative to the insect-overwintering theory. In one vicinity near Madison six cucumber fields on "new" land planted with seed from one source became diseased early, while four fields (planted with seed of a different source) on or very near land which had previously been planted with cucumbers did not develop the disease early. The early brood of beetles was fully as abundant on the four latter fields as on those six that became diseased early in the season.

SEED

The observations which formed the preliminary basis for the seed-overwintering theory have been printed before (*6*), but for the sake of bringing together all the pertinent evidence may be here repeated. In June, 1915, angular-leafspot was observed in abundance in a field south of Portsmouth, Va. The plants were developing their fifth and sixth leaves at the time. The field was on newly cleared land, surrounded by woods and at least 3 or 4 miles from the nearest cucumber patch. The evidence pointed strongly to the introduction of the organisms with the seed.

The developments in the fields near Madison in 1916 gave further evidence that the organisms are introduced with the seed. The six experimental fields previously mentioned were all on land which had not been planted to cucumbers for at least three years. Angular-leafspot appeared on seedlings in all six of these fields, and in three of them it was noted on the cotyledons. In the case of the four commercial fields near by which were planted with seed from another source the disease did not appear at all in one and not until late in the season in the other three. This evidence so strongly indicated that the bacteria live over winter on the seed that it seemed worth while to study the matter in the commercial seed fields. Accordingly the writer visited a large seed-producing center in Iowa and Dr. M. W. Gardner, because of his interest in the question in relation to cucumber anthracnose, visited a seed farm in Ohio. In one of the seed fields in Iowa the disease was widespread and, according to a hasty estimate, 25 per cent of the fruits were attacked. Dr. Gardner found spots on the fruits in the Ohio fields which he was reasonably sure were due to the angular-leafspot organism.

Since the fruit invasions are local and shallow, it is evident that the seed rarely, if ever, becomes attacked naturally. A study of the way the seed is thrashed, however, sheds further light on the way in which the seed may become contaminated.

The thrashing process practiced on the farms visited is probably in general use. It is begun by shoveling the whole fruits into a grinding machine which chops them up and allows the larger parts of the fruit pulp to be carried off on a rotating screen. The seed, the juice, and the smaller pieces of pulp fall through the screen and are drained into containers. This much of the process would doubtless afford ample opportunity for the organisms to reach the seed. The next step, however, probably increases the chances for the seed to become contaminated. The seed with the pulp and juice is left in the barrels with frequent stirring for a period of time varying usually from one to three days. The angular-leafspot organism doubtless multiplies rapidly in this well aerated mixture of juice and pulp unless conditions become unfavorable owing to the by-products of other organisms. After the material containing the seed has stood in the barrels for the time mentioned, it is poured into other containers and the seed separated out as well as possible by repeated washings with water. Then the seed is dried on shallow trays, at first in the sunlight and later indoors. The process of thrashing includes no step which would be likely to kill all the bacteria.

Seed for further study was sent to Madison from both the Iowa and Ohio farms. The details of some of the experiments performed with this seed and the results are here summarized.

EXPERIMENT OF FEBRUARY 20, 1917.—Sixteen flats of sand were steamed at 7 pounds' pressure for one hour. Then each flat was planted with approximately 150 seeds from Iowa. Before touching the seed the hands were rinsed in 70 per cent alcohol as a precautionary measure. After the flats were planted they were wet down with water that had been boiled (cooled), and boiled water was used in all subsequent watering. None of the resulting seedlings were diseased.

EXPERIMENT OF MARCH 3, 1917.[1]—Fourteen flats of sand were steamed as before. The hands were disinfected with mercuric chlorid and alcohol. The trowel was treated with hot water. Twelve flats were planted with seed from the lot from Ohio, about 100 seeds to each flat. One flat was planted with seed from the 1915 supply which had been treated with 1 per cent formaldehyde for 20 minutes and another with seed from the same lot which had been treated with 1 to 1,000 mercuric chlorid for five minutes—these two for controls. The flats were covered with sterilized wire screen to protect them from mice and rats. The flats were watered with water (cooled) which had been boiled.

On March 19 the writer found four seedlings in one of the flats showing typical signs of angular-leafspot as they had been observed on seedlings artifically infected by planting inoculated seed and on naturally infected seedlings in the field (Pl. 15, A). The attacked seedlings were in two separated places—two affected seedlings next to each other in each case—and apparently one seedling had been infected from its neighbor in each instance. From a seedling from each of the two places the organism was isolated and used in pure culture inoculations to reproduce the disease. Stained sections

[1]Performed by Dr. M. W. Gardner in connection with his work on cucumber anthracnose.

of one of the spots on one of the cotyledons showed bacteria in the intercellular spaces. Two of the seedlings were preserved as herbarium specimens. On March 27 another infected seedling was noted in another flat. The organism was isolated and identified by inoculation as before.

EXPERIMENT OF MARCH 27, 1917.[1]—Fourteen flats of sand and four of heavily composted garden soil were steamed for one hour at 7 pounds' pressure. All but two were planted with the seed from Ohio. These two planted with seed treated in 1916 with mercuric chlorid and untreated seed from the 1916 supply, respectively. Precautionary measures taken as before. On April 4 a typically infected seedling was noted in one of the sand flats planted with the Ohio seed and on April 7 a well-advanced stage of the disease was discovered on a seedling in another sand flat of the Ohio seed. There was no doubt as to the cause of the lesions from the characteristic signs—viz, water-soaked tissue and white exudate residue. Platings from each of these seedlings gave an abundance of the typical colonies.

The results of these experiments and the fact that in Dr. Gardner's later tests of the Ohio seed in sterile damp chambers one seedling in each of two damp chambers developed the typical signs of the disease prove that the angular-leafspot organisms may live for at least seven months on the seed. There seems no reason to doubt but that they can survive for two months longer and infect the seedlings as field observations have indicated.

The use of seed as badly contaminated as the lot from Ohio was found to be, would have resulted in the early development of angular-leafspot in as large a proportion of the fields as occurred in 1916 in Wisconsin. From the Ohio lot approximately 3,500 seeds were planted with the precautions described. Seven, or a proportion of 1 to 500, of the resulting seedlings developed angular-leafspot. With this proportion or 0.2 per cent and the use of 2 pounds of seed per acre, as is usually practiced, there would be about 72 plants infected from seed-borne organisms to every acre of cucumbers.

As to how the organisms are protected on the seed so as to withstand the long period of desiccation there is no conclusive evidence. It seems most likely to the writer, however, that they get in at the micropylar end of the seed, and so are protected within the seed coat. The fact that the infections of the seedlings nearly always occur on the edge of the cotyledons near the point of attachment to the stem—the part of the cotyledons which is at the micropylar end—indicates that the bacteria are probably harbored beneath the seed coat (Pl. 16, A). It might be argued that, since on germination the attached ends of the cotyledons are the first to emerge, the portion which becomes infected is the first part which is exposed to organisms on the surface of the seed. This explanation, however, seems less probable to the writer than that the organisms are sheltered inside the micropyle. At any rate subsequent work by Gardner and Gilbert (*8*) has shown that the bacteria are so located that they can be killed by chemical treatment of the seed.

[1] Performed in cooperation with Dr. M. W. Gardner.

REMEDIAL MEASURES

The matter of finding some means of controlling angular-leafspot has been kept in mind in all the studies, especially in comparing cucumber varieties as to susceptibility, in observing the ways in which the disease is spread, in testing the sensitiveness of the organism to desiccation, to heat and chemical germicides, and in trying to determine how the bacteria are overwintered.

RESISTANT VARIETIES

Tests made in the field in 1915 and 1916 by growing the horticultural varieties (listed on page 206) where they were exposed to infection yielded no encouraging results. There was no marked difference in susceptibility between the varieties. No instance of individual resistance has been observed in all the fields which have been examined.

SANITATION

The evidence recorded under the section on dissemination by pickers justifies the recommendation that where feasible the picking of fields into which the disease has been introduced be done at times other than in early mornings or on rainy days when the bacterial exudate is abundant. In cases where it is necessary to pick over a partly diseased field under those unfavorable conditions it may be worth while to pick the healthy part of the field first.

The hope for the complete control of the insect pests, particularly the cucumber beetles, seems to be a thing for which there is little basis. The fact, however, as discussed under the consideration of dissemination, that there is good evidence that these insects are instrumental in spreading the disease from one field to another makes more urgent the need of finding better ways of holding them in check.

SPRAYING

Spraying experiments in which Bordeaux mixture (3–6–50) was the principal fungicide used, were under observation in Wisconsin during the summers of 1914, 1915, and 1916. Noticeable checking of the disease resulted each year. Yield results were in all cases so vitiated by factors other than the spraying, especially the mosaic disease, that comparisons of them were of little value. Furthermore, the disease did not develop in the most destructive way on the experimental fields. The data at hand therefore hardly justify a definite statement of the value of spraying for this disease, but, in the opinion of the writer, the practice would not in Wisconsin and neighboring States be generally profitable on a commercial scale. Several reasons have furnishd the basis for this conclusion. Because of the early appearance of the disease, spraying, to be most effective, would have to be started nearly as soon as the plants came up. Because of this need for beginning early and continuing the

spraying at frequent intervals throughout the season, the cost would probably be greater than could be compensated by the resulting increase in yield. Cucumber vines normally grow so rapidly that the intervals between spraying would have to be short in order that a considerable portion of the younger leaves would not be exposed to infection a good deal of the time. The fact, however, that the disease is mainly dependent on rain for dissemination and that long, rainless periods occur at irregular times would make it a hard matter to recommend a spraying schedule which would be economical.

Spraying where profitable because of other considerations has no doubt been of increased value because of the partial protection afforded from angular-leafspot damage.

Burger (*4*) reported beneficial results from spraying for this disease on the basis of a limited amount of spraying in one season. He found a decidedly smaller number of infected fruits in the sprayed than in the unsprayed plots, and reported that the leaves in the sprayed plots were healthier than those in the check rows. It is interesting to note, however, that his recorded yields show that in every case the total yield, including infected and healthy fruits, was greater from the check than from the sprayed plot. This fact may be correlated with the unsettled question of spray injury to cucumber.

The readiness with which angular-leafspot is spread by spattering of rain makes a spraying experiment, in which the check rows are parallel and adjacent to those sprayed, incomparable to the spraying of a whole field. This fact should be borne in mind when further spraying tests are made.

SEED TREATMENT

The evidence indicates strongly that the angular-leafspot organism overwinters principally on the seed. If this be true, the matter of controlling the disease is greatly simplified, especially from the standpoint of the industry of growing cucumbers for pickling. Some of the pickle companies grow their own seed, while others buy seed from seedsmen. All companies, so far as is known to the writer, furnish the seed to the growers with whom they contract to raise the cucumbers. There will be little difficulty, therefore, in getting the seed disinfected before it is distributed to the farmers, after a satisfactory method of treatment has been worked out.

Preliminary tests of treatments with hot water and with chemical disinfectants have been made. Seed has been treated as follows: Soaked in water at 50° and 52° C. for 10 minutes; in formalin (4 per cent) for 5 minutes and 2 minutes; in copper sulphate (1 per cent) 10 minutes and 5 minutes; and in mercuric chlorid (1 : 1,000) for 5 minutes and 2 minutes. These tests were run on such a small scale because of limited greenhouse space for testing germination that conclusions can not be drawn as to

the effectiveness of the treatments in killing the causal organism, but they do indicate that no important injury [1] to the seed from these treatments may be expected. Extensive field tests with treated seed and further field trials of disinfectants with special reference to injury to the seed are under way in Wisconsin, Michigan, and Indiana under the supervision of Mr. W. W. Gilbert and Dr. M. W. Gardner.

SUMMARY

Angular-leafspot of cucumber was first noted in Wisconsin in 1914 and its bacterial nature established in 1915. The disease is the same as that described by Smith and Bryan (*15*) and earlier reported by Burger (*2*), Traverso (*16*), and Potebnia (*12*).

The disease is probably world-wide in its distribution. Under favorable meteorological conditions it does a good deal of damage. Because of its widespread and frequent occurrence it should be ranked among the cucumber diseases of major economic importance.

Leaf infection is stomatal. Inoculations made at different hours showed that infection occurs chiefly during the day rather than the night. This is probably to be explained by the fact that the stomata are open during the day and closed at night.

Fruit infection is stomatal. The disease first appears there as small, localized, circular, water-soaked spots. The centers of the spots later become whitened, so that they are more readily noticed.

Rain is the most important means of dissemination, but pickers and probably insects play a part in this process.

The causal organism is sensitive to desiccation, is readily killed in artificial media by freezing, is killed in liquid media by an exposure for 10 minutes at 50° C., and is readily killed by dilute solutions of formaldehyde, copper sulphate, or mercuric chlorid. The sensitiveness of the organism to these chemicals is increasingly greater in the order mentioned.

There is substantial evidence that the causal bacteria overwinter with the seed.

No marked variation in resistance or susceptibility has been found among horticultural varieties of cucumbers. A few ornamental gourds are attacked by the disease. Attacks are limited to the cucurbits, and in that family no important crop plant other than the cucumber has been found affected.

Sanitary measures, such as precautions in picking and in control of insects, may be helpful. Spraying with Bordeaux mixture checks the disease, but is of doubtful value as a general commercial procedure in regions where spraying would not otherwise be practiced. Seed treatment offers the greatest hope of satisfactory control.

[1] In the subsequent field tests carried on by Gilbert and Gardner, the 4 per cent formalin treatment caused considerable injury to cucumber seedlings, resulting in marked rolling of cotyledons and retardation of growth. The mercuric chlorid treatment (1: 1,000 for five minutes) has proved safe and effective (*8*).

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PLATE 13

A.—Cucumber leaf five days after inoculation with *Bacterium lachrymans*, showing severe infection. The dark, angular spots had a water-soaked appearance. Drops of bacterial exudate may be seen on some of the spots. Photographed by Mr. Fred R. Jones.

B.—Plant a, photographed seven days after inoculation with *Bact. lachrymans* shows considerable stunting as compared with the uninoculated control, plant b. Young plants as severely attacked have often been seen in the field.

A
a
B
b

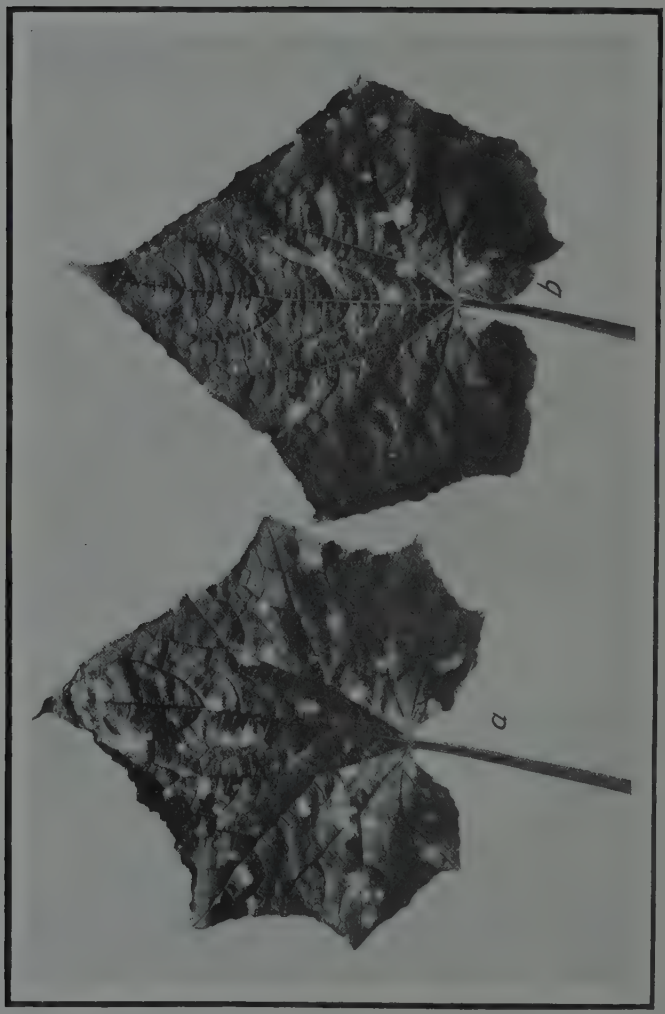
b
a

PLATE 14

Stomatal movement in relation to infection. The cucumber leaves used in the experiment were of the same age and on similar plants. So far as possible, conditions of inoculation were similar except that leaf a was inoculated with *Bacterium lachrymans* at 9.15 a. m. and leaf b at 6 p. m.

PLATE 15

A.—Overwintering on seed: Natural infections on cotyledons of seedling grown in steamed sand from commercial seed which had been kept in storage for seven months after harvesting. Experiment of March 3, 1917. Enlarged about 1½ times.

B.—Picker dissemination. Infection resulting from inoculation of a cucumber leaf at 7.30 a. m. by rubbing with the hand immediately after touching diseased exudate-bearing leaves.

C.—Dissemination by rain. The older leaves in the center of the row were badly infected during a rainy period. Young leaves on the sides of the row which developed during a rainless period are comparatively free from the disease. Photographed by Mr. W. W. Gilbert.

B

a
b
A
B

PLATE 16

A.—Seedling infection resulting from seed inoculation with *Bacterium lachrymans*. Seeds were wet with a pure culture of the angular-leafspot organism and planted in sterilized soil. Note location of cotyledon infections. Photographed 14 days after planting.

B.—Cucumber fruit showing small, watersoaked, circular spots with white centers resulting from natural infections with angular-leafspot.